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(71) Applicants and
(72) Inventors: **ZHAO, Jian** [CN/CN]; 420 Jiang Ning Road, 25E, Shanghai 200041 (CN). **CHEN, Hu** [CN/CN]; 420 Jiang Ning Road, 25E, Shanghai, 200041 (CN). **JIN, Bei, Wen** [CN/CN]; 420 Jiang Ning Road, 25E, Shanghai, 200041 (CN). **ZHOU, Rui** [CN/CN]; 420 Jiang Ning Road, 25E, Shanghai 200041 (CN).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **WOUTERS, Bert,** C. [BE/BE]; Sysmans, Graaf, J. de Pret Staat 15, B-2900 Schoten (BE).

(74) Agent: **SHOUSE, Emily, E.**; Waddey & Patterson, 414 Union Street, Suite 2020, Nashville, TN 37219 (US).

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(54) Title: METHOD OF OBTAINING A TEA PIGMENT FROM TEA LEAVES

(57) Abstract: The method according to the invention relates to a method of obtaining a tea pigment comprising theaflavin, thearubigin, theabrownin and catechins, characterised by (1) mixing tea leaves and ethanol, soaking and refluxing the obtained suspension; (2) centrifuging the suspension and discarding the pellet; (3) adding the remaining samples to a gel filtration column, washing the column; and (4) collecting the washing solution, extracting the tea pigment using a halogenated hydrocarbon having 1-3 carbon atoms, discarding the water phase, evaporating the halogenated hydrocarbon, and recovering a tea pigment powder containing less impurities and showing higher efficacy. The extraction product is applicable in the field of applications against hyperlipidemia and related diseases.

Method of obtaining a tea pigment from tea leaves

Description of the invention

The present invention provides a method of obtaining a tea pigment from tea leaves. The obtained tea pigment comprises theaflavin, thearubigin, theabrownin and catechins.

Detailed description of the invention

The invention relates to a method comprising the following steps:

(1) mixing tea leaves and thanol, soaking and refluxing the obtained suspension;
(2) centrifuging the suspension and discarding the pellet;
(3) adding the remaining samples to a gel filtration column, washing the column; and
(4) collecting the washing solution, extracting the tea pigment using a halogenated hydrocarbon having 1 – 3 carbon atoms, discarding the water phase, evaporating the halogenated hydrocarbon and recovering a tea pigment powder containing less impurities and showing higher efficacy.

More in particular the method according to the invention comprises the following steps:

(1) mixing tea leaves and ethanol in a w/w – ratio of 1:1-20, preferably about 1:10, soaking and refluxing the obtained suspension;
(2) centrifuging the suspension and discarding the pellet;
(3) adding the samples to a Sephadex column, washing the column; and
(4) collecting the washing solution, extracting tea pigment using a chlorinated hydrocarbon having one carbon atom, discarding the water phase, evaporating the chlorinated hydrocarbon and recovering a tea pigment powder containing less impurities and showing higher efficacy.

Most preferably the method according to the invention is carried out in the following way:

1. Mixing tea and 80% ethanol solution (w/w = 1:10), soaking the suspension at room temperature for 2 hours, heating and refluxing the suspension for 1 hour, filtrating the tea extraction solution, eliminating the tea residue; and

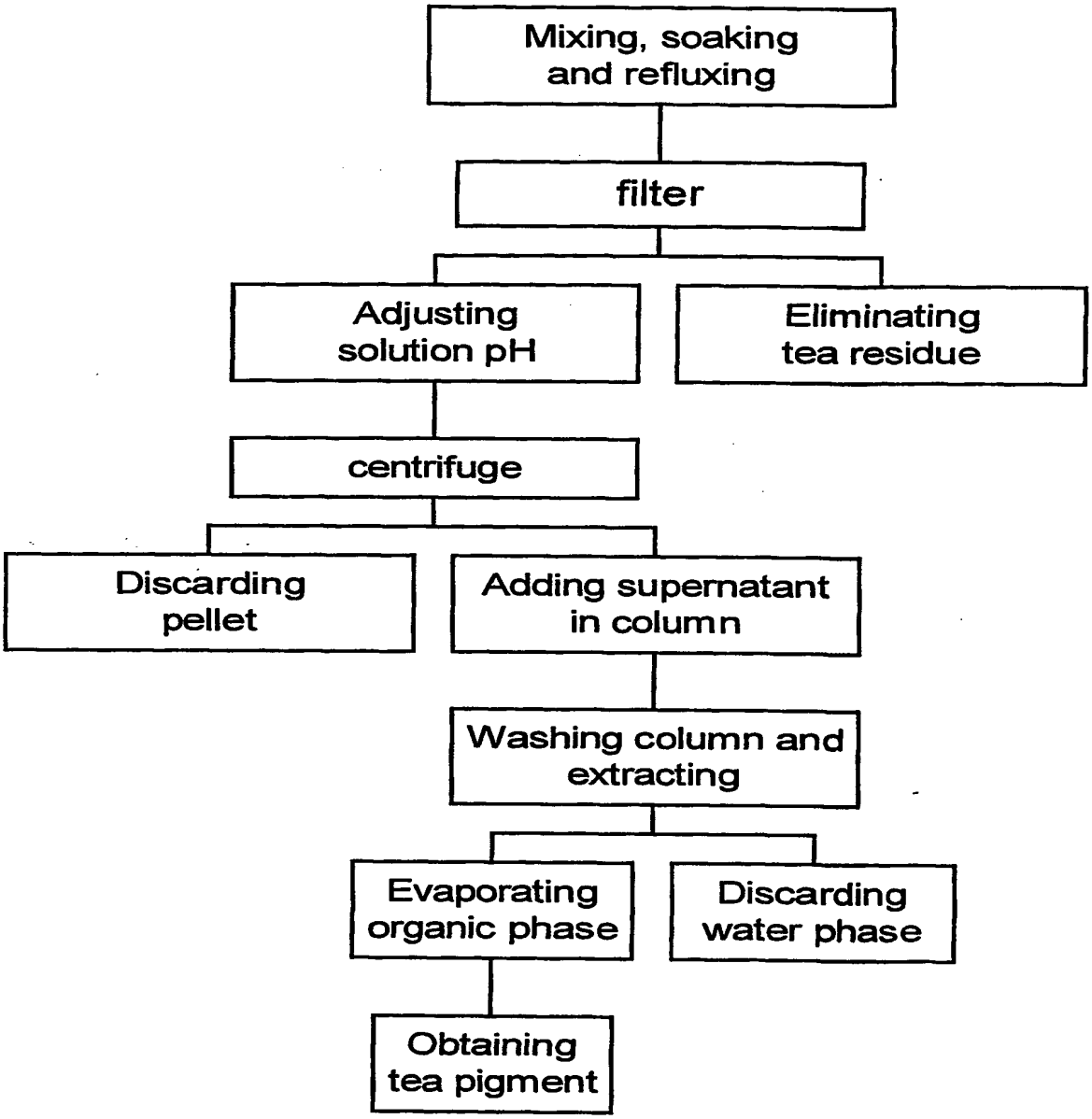
2. adjusting the suspension pH to 3.2, using 1mol/L HC1, 10,000 rpm centrifuge for 30 min, discarding the pellet; and
3. adjusting the supernatant solution pH to 7.0, using 1mol/L NaOH, adding the sample to a Sephadex LH-20 column, washing the column with 40% - 100% ethanol solution; and
4. collecting the washing solution, adjusting the washing solution pH to 8.0, using 1mol/L NaOH, extracting tea pigment using CH_2C1_2, discarding the water phase, evaporating the CH_2C1_2 and obtaining a tea pigment powder having excellent pharmaceutical properties, in particular in applications against hyperlipidemia and related diseases.

Further the invention relates to the product obtained by means of the method defined above.

Another aspect of the convention relates to the pharmaceutical compositions comprising the product obtained by means of method defined above. The pharmaceutical composition is applicable in the field of applications against hyperlipidemia and related diseases.

Figure

The production process according to the invention is illustrated by the following figure.



Experimental Studies and Clinical Application

1. Animal experiments

(1) Safety test

Tea pigment obtained according to the method of the invention is poured directly into rats' stomachs. The dose for rats is 100 times higher than that for human. Observing these rats' behaviour for one week, we find that rats act normally. This test shows the tea pigment according to the invention is safe.

(2) Effect on serum lipids

Tea pigment according to the invention can induce decrease of serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), and raise high density lipoprotein cholesterol (HDL) in comparison with control groups.

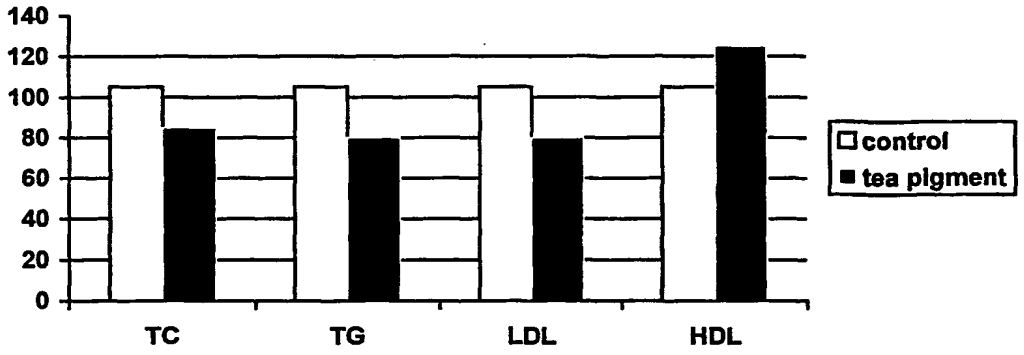


Figure 2. effects of tea pigment on serum lipids

2. Population Studies

(1) Hyperlipidemia study

The following values of TC, TG, HDL, LDL are the standard for normal human:

TC	3.1	- 5.7mol/L
TG	0.56	- 1.7mol/L
HDL	1.04	- 1.55mol/L
LDL	1.80	- 3.36mol/L

A total of 1,696 patients participated in this trial, 920 males and 776 females, ages 35 to 81 with an average weight of 58.9 ± 7.9. Patients took 125 mg tea pigment 3 times/day for 4 weeks.

Patients satisfied the following criteria,

1) did not suffer an acute heart attack, brain damage, no injury, no operations within the last 6 months;
2) no kidney diseases;
3) no diabetes mellitus;
4) no thyroid disease;
5) no phase III hypertension;
6) no drug induced hyperlipidemia;
7) no pregnant women.

Table 1 changes in blood TC, TG, HDL and LDL levels.

	Cases (n)	Before treatment (mol/L)	After treatment (mol/L)	Rate of change (%)	P value
TC	811	6.71 + 0.55	5.65 + 0.41	- 15.8	< 0,01
TG	923	2.95 + 0.59	2.27 + 0.31	- 23.1	< 0,01
LDL	154	4.05 + 0.37	3.35 + 0.34	- 17.3	< 0,01
HDL	276	1.19 + 0.28	1.34 + 0.19	+ 12.6	< 0,01

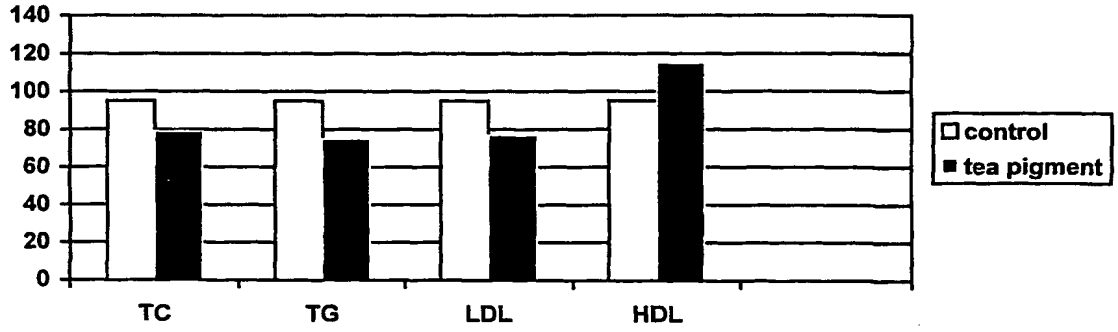


Fig. 3 changes in patients blood TC, TG, HDL and LDL levels

Table 2 shows changes on patients with hyperlipidemia.

	Cases (n)	Markedly Improved (%)	Improved (%)	Not improved (%)	Total improvement
TC	811	59.8 (485)	12.5 (101)	27.7 (225)	72.3
TG	923	41.7 (385)	24.1 (222)	34.2 (316)	65.8
LDL	154	48.7 (75)	31.2 (48)	20.1 (31)	79.9
HDL	276	50.7 (140)	23.9 (66)	25.4 (70)	74.7

(2) Dose-Effect and Period of Treatment-Effect Relationships:

250mg versus 125mg Tea pigment

60 days versus 30 days of Tea pigment treatment.

A total of 521 patients participated in this trial, 310 males and 211 females, ages 28 to 79 with an average weight of 55.2 ± 5.9.

Group A: 125 mg 3 times a day

Group B: 250 mg 3 times a day

Patients satisfied the following criteria,

1) did not suffer an acute heart attack, brain damage, no injury, no operations within last 6 months;
2) no kidney diseases;
3) no diabetes mellitus
4) no thyroid disease
5) no phase III hypertension
6) no drug induced hyperlipidemia
7) no pregnant women.

Table 3 shows changes in blood TC, TG, HDL and LDL levels. The period of treatment is 30 days.

	Group	Cases (n)	Before treatment (mol/L)	After treatment (mol/L)	Rate of change (%)	P value
TC	A	210	6.51 ± 0.97	5.51 ± 0.76	-15.3	<0,01
TC	B	156	6.54 ± 0.88	5.21 ± 0.61	-20.3	<0,01
TG	A	198	3.11 ± 0.74	2.34 ± 0.46	-24.8	<0,01
TG	B	112	3.06 ± 0.78	2.01 ± 0.71	-34.3	<0,01
LDL	A	165	4.32 ± 0.76	3.41 ± 0.59	-21.1	<0,01
LDL	B	120	4.26 ± 0.81	3.03 ± 0.65	-28.9	<0,01
HDL	A	171	1.04 ± 0.35	1.23 ± 0.29	+18.3	<0,01
HDL	B	104	0.98 ± 0.26	1.31 ± 0.22	+33.7	<0,01

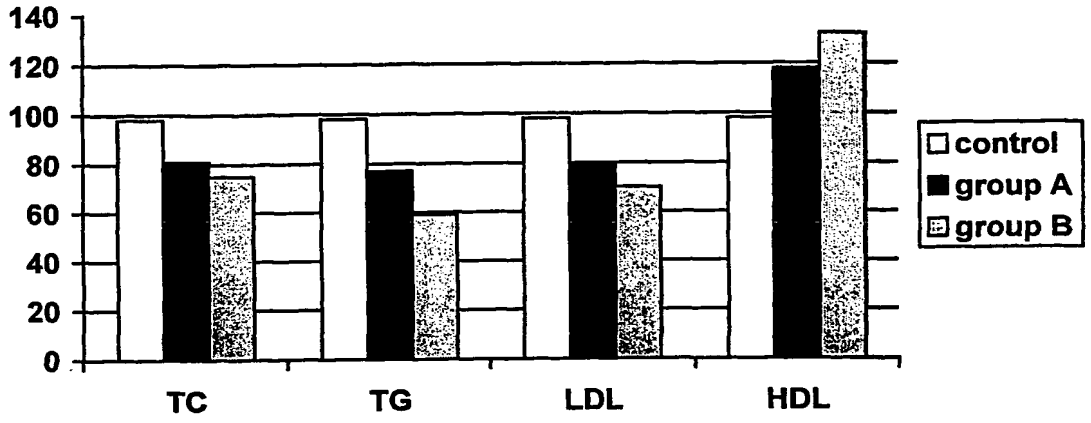


Fig. 4 changes in patient's blood TC, TG, HDL and LDL levels (30 days)

Table 4 shows changes on patients with hyperlipidemia

The period of treatment is 30 days.

	Group	Cases (n)	Markedly Improved (%)	Improved (%)	Not Improved (%)	Total improvement %
TC	A	210	55.2 (116)	15.2 (32)	29.6 (62)	70.4
TC	B	156	62.8 (98)	14.1 (22)	23.1 (36)	76.9
TG	A	198	39.9 (79)	21.2 (42)	38.9 (77)	61.1
TG	B	112	50.9 (57)	19.6 (22)	29.5 (33)	70.5
LDL	A	165	47.3 (78)	28.5 (47)	24.2 (4)	75.8
LDL	B	120	50.8 (61)	30.0 (36)	19.2 (23)	81.8
HDL	A	171	49.7 (85)	21.6 (37)	28.7 (49)	71.4
HDL	B	104	55.8 (58)	24.0 (25)	20.2 (21)	79.8

Table 5 shows changes in blood TC, TG, HDL and LDL levels. The period of treatment is 60 days.

	Group	Cases (n)	Before treatment (mol/L)	After treatment (mol/L)	Rate of change (%)	P value
TC	A	210	6.51 ± 0.97	5.48 ± 0.73	-15.8	<0,01
TC	B	156	6.54 ± 0.88	5.14 ± 0.75	-21.4	<0,01
TG	A	198	3.11 ± 0.74	2.30 ± 0.46	-26.0	<0,01
TG	B	112	3.06 ± 0.78	1.92 ± 0.53	-37.2	<0,01
LDL	A	165	4.32 ± 0.76	3.38 ± 0.46	-21.8	<0,01
LDL	B	120	4.26 ± 0.81	2.89 ± 0.58	-32.2	<0,01
HDL	A	171	1.04 ± 0.35	1.24 ± 0.24	+19.2	<0,01
HDL	B	104	0.98 ± 0.26	1.34 ± 0.19	+36.7	<0,01

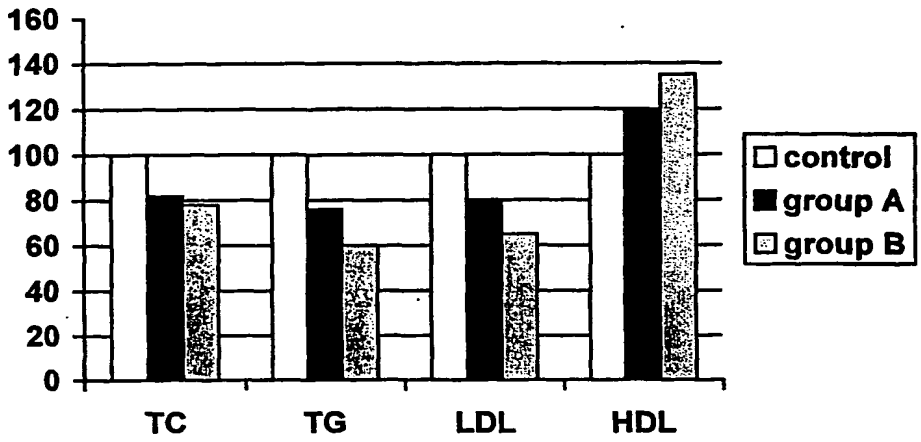


Fig. 5 changes in atients' blood TC, TG, HDL and LDL levels (60 days).

Table 6 shows changes on patients with hyperlipidemia.

The period of treatment is 60 days.

	Group	Cases (n)	Markedly Improved (%)	Improved (%)	Not Improved (%)	Total improvement %
TC	A	210	59.0 (124)	12.4 (26)	28.6 (60)	71.4
TC	B	156	67.9 (106)	10.3 (16)	21.8 (34)	78.2
TG	A	198	46.5 (92)	17.6 (35)	35.9 (71)	64.1
TG	B	112	59.8 (67)	13.4 (15)	26.8 (30)	73.2
LDL	A	165	57.0 (94)	20.0 (33)	23.0 (38)	77.0
LDL	B	120	65.0 (78)	18.3 (22)	16.7 (20)	83.3
HDL	A	171	54.4 (93)	18.1 (31)	27.5 (47)	72.5
HDL	B	104	59.6 (62)	21.2 (22)	19.2 (20)	81.8

Significant differences occurred in all lipid level measurements.

However, no significant differences ($P>0.05$) occurred in the same dose and 2 periods of treatment.

Claims

1. Method of obtaining a tea pigment comprising theaflavin, thearubigin, theabrownin and catechins, characterised by
   (1) mixing tea leaves and ethanol, soaking and refluxing the obtained suspension;
   (2) centrifuging the suspension and discarding the pellet;
   (3) adding the remaining samples to a gel filtration column, washing the column; and
   (4) collecting the washing solution, extracting the tea pigment using a halogenated hydrocarbon having 1-3 carbon atoms, discarding the water phase, evaporating the halogenated hydrocarbon, and recovering a tea pigment powder containing less impurities and showing higher efficacy.
2. Method according to claim 1, characterised in that in step (1) the tea leaves and the ethanol are mixed in a w/w-ratio of 1:1-20.
3. Method according to claim 2, characterised in that in step (1) the tea leaves and the ethanol are mixed in a w/w-ratio of 1:10.
4. Method according to any of claims 1-3, characterised in that in step (3) the samples are added to a Sephadex column, preferably a Sephadox LH-20 column.
5. Method according to any of the claims 1-4, characterised in that in step (4) the halogenated hydrocarbon is a chlorinated hydrocarbon having one carbon atom.
6. Method according to claim 5 characterised in that in step (4) the halogenated hydrocarbon is CH_2Cl_2.
7. The product obtained by carrying out the method according to any of the claims 1-6.
8. A pharmaceutical composition, comprising the product according to claim 7 as one of the active components.

Fig 1

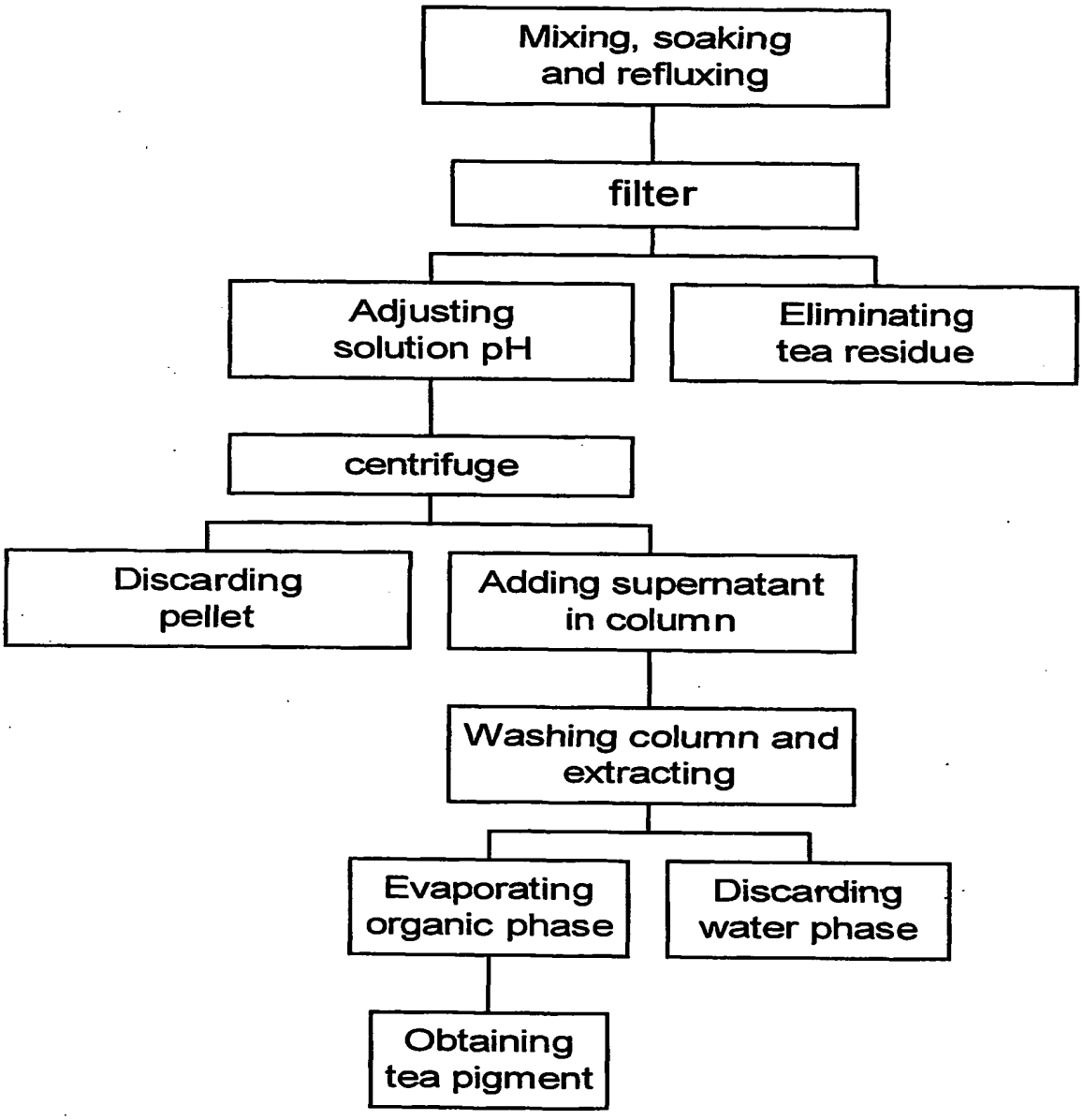


Fig 2

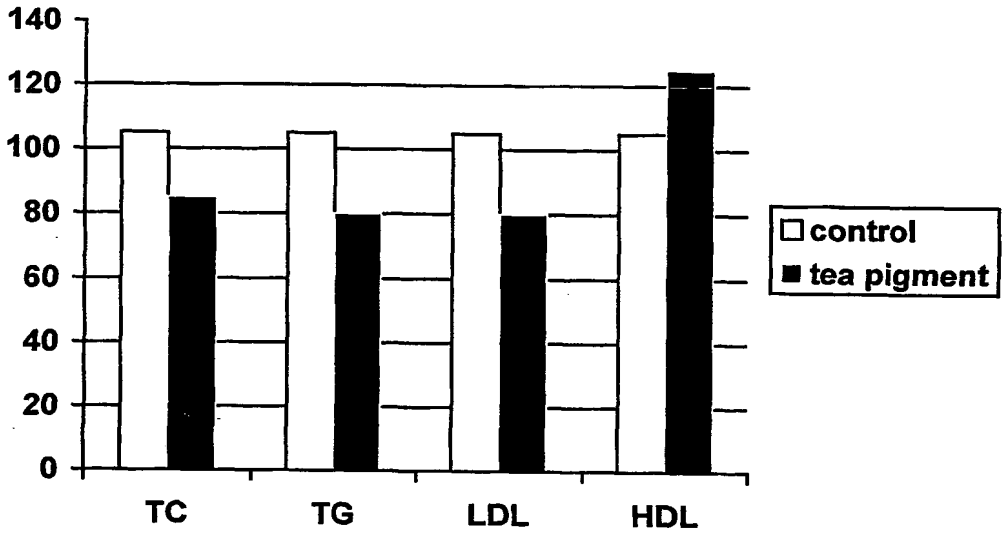


Fig 3

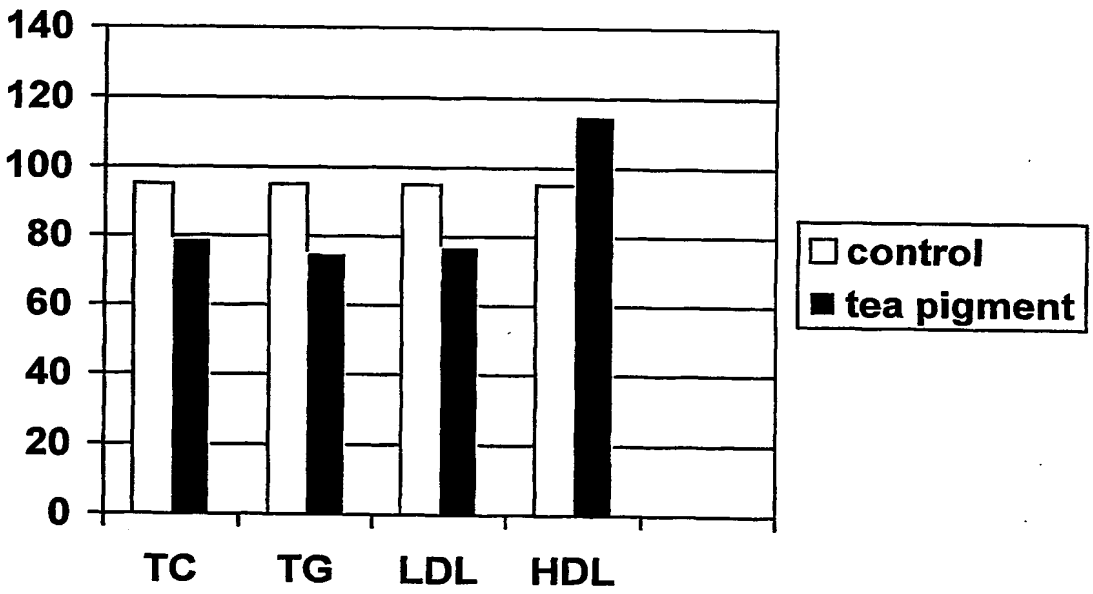


Fig 4

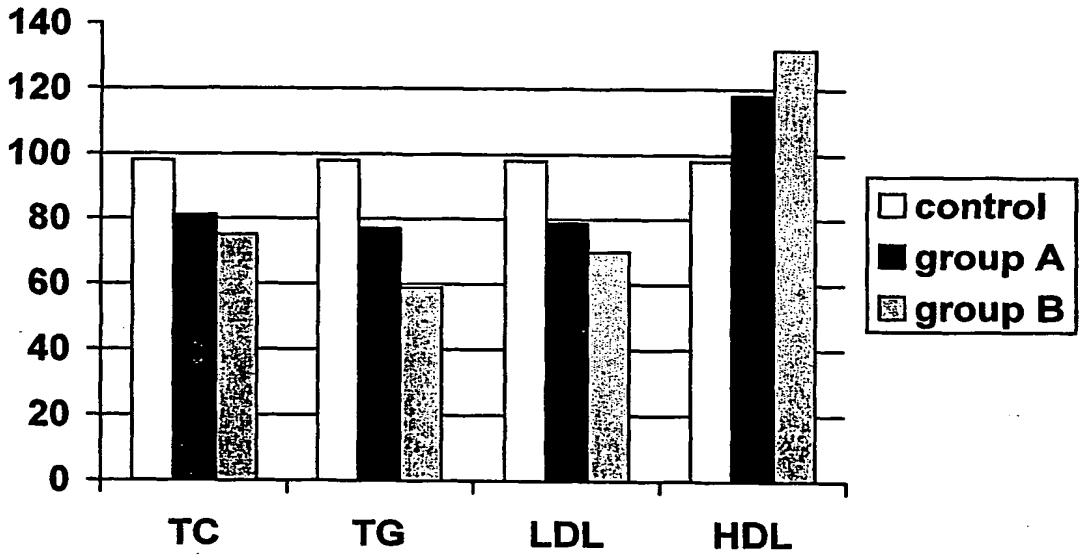
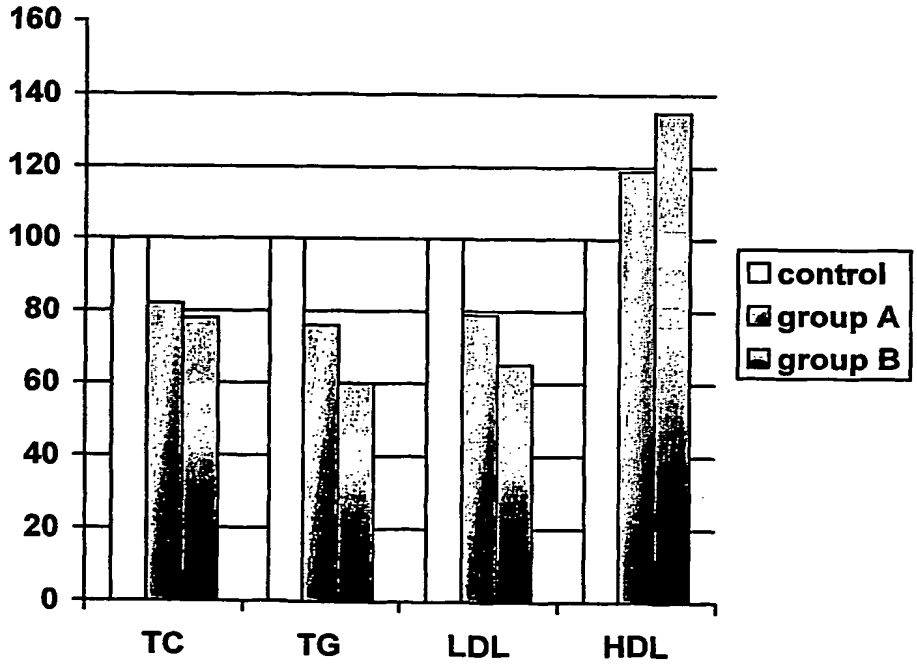


Fig 5



INTERNATIONAL SEARCH REPORT

	National Application No PCT/EP 00/05659

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, FSTA, MEDLINE, PASCAL, CAB Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 613 672 A (HARA YUKIHIKO) 23 September 1986 (1986-09-23) column 2, line 20 -column 3, line 59	1-8
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1992 HAYASHI M ET AL: "INHIBITORY EFFECTS OF GREEN TEA EXTRACTED WITH WATER OR ETHANOL ON SERUM LIPIDS OF RATS TREATED WITH TRITON WR-1339" Database accession no. PREV199294077291 XP002164188 abstract & PHARMACOMETRICS, vol. 43, no. 6, 1992, pages 555-559, ISSN: 0300-8533	1,8
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2001	20/07/2001

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/05659

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 198711 Derwent Publications Ltd., London, GB; Class B04, AN 1987-076886 XP002164189 & JP 62 030711 A (FURUKAWA NETSUGAKE), 9 February 1987 (1987-02-09) abstract	1,8

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INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
PCT/EP 00/05659

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